Theses of the PhD Dissertation

Genetic Diversity, Host Adaptation Signals, and the Spread of Antimicrobial Resistance in *Pasteurella multocida*

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1. Background and Objectives of the Doctoral Dissertation

Pasteurella multocida is a globally distributed Gram-negative bacterial species with zoonotic potential and considerable economic and public health significance. The strains of this species exhibit marked genotypic and phenotypic heterogeneity, contributing to the development of diverse disease manifestations with varying pathogenic mechanisms across different host species. Infected animals are typically treated with antibiotics; however, the increasing emergence and spread of multidrug-resistant (MDR) strains represent a growing challenge for both veterinary and human medicine. Detailed molecular characterization of the strains, together with a deeper understanding of their antimicrobial resistance, is essential for elucidating the pathogenesis and epidemiological dynamics of infections, improving therapeutic strategies, and reducing zoonotic risks. Collectively, these findings contribute to the development of more targeted

prevention and treatment approaches.

The aim of our study was the detailed phenotypic and genotypic characterization of *P. multocida* strains. We investigated and statistically analysed the relationships among virulence gene profiles (VGPs) determined based on virulence gene patterns, clusters established according to ompA gene sequences, and other characteristics of the strains, including serotype, Multi-host (MH) and Rural Industries Research and Development Corporation (RIRDC) MLST sequence types (STs), as well as host origin. Furthermore, our objective was to assess the antimicrobial susceptibility profiles *P. multocida* strains of Hungarian and French origin using both phenotypic (disk diffusion and broth microdilution methods) and genotypic (whole-genome sequencing) approaches.

2. Material and methods

In this study, the antimicrobial susceptibility of 80 *P. multocida* isolates of Hungarian and French origin, collected from different host species between 2004 and 2023, was investigated. Particular attention was paid to the occurrence of MDR strains and to the identification of resistance-related trends. The isolates were tested against antibiotics belonging to ten antimicrobial classes, including penicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, fluoroquinolones, lincosamides, phenicols, polymyxins, and sulfonamides. Sequences of antimicrobial resistance genes and single nucleotide polymorphisms were identified using the Bacterial and Viral Bioinformatics Resource Center and the Resistance Gene Identifier tool of the Comprehensive Antibiotic Resistance Database. Plasmid carriage of MDR strains was analysed using the Solu server. To increase the reliability of the statistical analyses, detailed genotypic characterization was additionally performed on 50

P. multocida genomes available in the National Center for Biotechnology Information database. During the study, strain sequences were aligned to different reference genomes, allowing the determination of virulence gene carriage and the identification of additional VGP groups. Capsular types and lipopolysaccharide (LPS) genotypes were determined using a Danish bioinformatics server, while MH- and RIRDC-STs were identified using the Public Databases for Molecular Typing and Microbial Genome Diversity platform.

3. Main Results and Conclusions

A phylogenetic tree was constructed based on the *omp*A gene sequences of the strains, in which the isolates formed twenty-five well-separated clusters. Fourteen novel virulence gene profiles (VGPs) were identified based on virulence gene patterns, increasing the currently known number of VGP groups to twenty-seven. The most common capsule–LPS genotype combination was A:L3, followed by A:L1 and F:L3. Several strains carried point mutations within the *tad*D primer-binding region, resulting in false-negative polymerase chain reaction (PCR) results. To overcome this limitation, new primers were designed based on whole-genome sequences, enabling reliable diagnostic detection of the gene.

Phenotypic antimicrobial susceptibility testing demonstrated that cephalosporins and phenicols were the most effective antimicrobial classes against *P. multocida*. In addition, most strains showed high susceptibility to fluoroquinolones and tetracyclines. In contrast, high resistance rates were

observed against sulfamethoxazole and clindamycin.

The comprehensive genomic and phenotypic approach of the present study provided new insights into the population genetic structure, host adaptation, and antimicrobial resistance profile of *P. multocida.* Our results and statistical analyses demonstrated at multiple levels that the remarkable diversity of *P. multocida* is not the consequence of isolated genetic factors, but rather the result of complex interactions among virulence gene patterns, sequence types, ompA clusters, and host-specific adaptive pathways.

The identified VGP groups showed strong correlations with host species, subspecies, capsular type, LPS genotype, and both MH- and RIRDC-STs, suggesting that these groups represent evolutionarily relevant genetic patterns rather than simple statistical clusters.

Furthermore, the close association observed between *omp*A clusters, VGPs, sequence types, and host origin indicates that *omp*A variability

reflects complex biological and epidemiological characteristics of the strains.

The difficulties encountered during PCR-based detection of the *tad*D gene further emphasize the importance of WGS-based analyses.

Antimicrobial susceptibility profiles indicate that phenicols and cephalosporins have remained among the most effective therapeutic options for the treatment of pasteurellosis, while most strains also retained high susceptibility to fluoroquinolones and tetracyclines. However, the decreasing susceptibility observed in the case of fluoroquinolones is of concern. Enrofloxacin is considered a critically important antimicrobial agent, widely used in both veterinary and human medicine, and the observed decline in susceptibility is likely associated with excessive or inappropriate antimicrobial use.

High resistance rates were observed against clindamycin and sulfamethoxazole; therefore, the therapeutic use of these compounds is not recommended, although in the latter case genotypic

confirmation of resistance was detected only at a low frequency.

The genetic background of the phenotypic resistance observed against β-lactams and macrolides remains unclear and requires further investigation.

Statistical comparison of phenotypic susceptibility testing and WGS-based ARG detection indicated that the different methods identify resistance with varying sensitivity. The stronger correlation observed between MIC values and ARG profiles suggests that genotypic results are more closely associated with quantitative susceptibility measurements. An exception was observed in the case of tetracyclines, where disk diffusion results reflected resistance gene carriage more accurately.

4. Novel Scientific Findings of the Dissertation

1. For the first time in Hungary, whole-genome sequencing of a large collection (n = 80) of *Pasteurella multocida* strains originating from twelve different animal species was performed using the Illumina MiSeq platform.

2. By analysing the whole-genome sequences of our isolates together with additional publicly available genomes from the NCBI database, the virulence gene patterns of a total of 130 *P. multocida* isolates were characterized. During this work, fourteen novel virulence gene profiles were identified, expanding the previously established classification system of our research group. Avian-origin strains were included in these profiles for the first time. As a result of our work, a total of twenty-seven virulence gene profiles are currently available for the characterization of P. multocida strains.

3. A novel primer pair targeting the *tad*D virulence gene was designed, amplifying a 445 bp

PCR product. The newly designed primers enabled the detection of the gene in strains that had previously yielded false-negative PCR results, thereby improving the reliability of diagnostic identification.

4. A phylogenetic tree was constructed based on the *omp*A gene sequences of the strains, in which the isolates formed twenty-five distinct clusters. For the first time, associations were identified among the characteristics of strains belonging to the same cluster.

5. During the determination of Multi-host and RIRDC MLST sequence types, a total of sixteen novel sequence types (MH: 6; RIRDC: 10) were identified. Our analyses revealed associations among sequence types, virulence gene profiles, and *omp*A gene sequences.

6. Phenotypic and genotypic antimicrobial susceptibility profiles were determined for a large collection of Hungarian and French *P. multocida* strains isolated from various animal species, and the performance of phenotypic susceptibility testing

methods was evaluated in relation to genotypic results.

5. Publications Related to the Dissertation

5.1. Publications in Peer-Reviewed Scientific Journals with Impact Factor

1. Pintér, K., Kerek, Á., Magyar, T., 2023.: **A *Pasteurella multocida* törzsek antibiotikumérzékenysége, az antimikrobiális rezisztencia genetikai háttere, Irodalmi összefoglaló**, Magyar Állatorvosok Lapja, 145 (4), 239-256., IF: 0,2

2. Pintér, K., Pollák, B. D., Gantelet, H., Magyar, T., 2024.: **Antimicrobial susceptibility of *Pasteurella multocida* strains isolated from different host species**, Acta Veterinaria Hungarica, 72 (2), 71-79., IF: 0,7

3. Erdélyi, K., Pintér, K., Pollák, B. D., Bakcsa, E., Szeredi, L., Lehotzky, P., Magyar, T., 2025.: **Emergence of hemorrhagic septicemia caused by MLST type ST64 *Pasteurella multocida* in an European fallow deer population in Hungary,** Journal of Veterinary Diagnostic Investigation, 37 (3), 467-470., IF: 1,2

4. Pintér, K., Domán, M., Wehmann, E., Makrai, L., Gantelet, H., Magyar, T., 2025.:

Correlations between virulence gene profiles and other genetic factors of *Pasteurella multocida* strains isolated from various host species, Veterinary Microbiology, 308 (2025), 110657., IF: 2,7

5. Pintér, K., Domán, M., Wehmann, E., Gantelet, H., Magyar T., 2025.: **Comparative analysis of phenotypic and genotypic antibiotic susceptibility of *Pasteurella multocida* Isolated from various host species in France and Hungary,** Antibiotics, 14 (9), 906., IF: 4,6

5.2. Conference Posters and Presentations

1. Pintér, K., Pollák, B. D., Makrai, L., Magyar, T.: **Különböző állatfajokból izolált *Pasteurella multocida* törzsek fenotípusos antibiotikum-érzékenységi vizsgálata**, II. Magyar Agrártudományi Doktoranduszok Szimpóziuma, Budapest, 2024. 02.16.

2. Pintér, K., Pollák, B. D., Wehmann, E., Makrai, L., Solymosi, N., Magyar, T.: ***Pasteurella multocida* törzsek genotípusos jellemzése**,

XXVII. Tavaszi Szél Konferencia, Budapest, 2024. május 3-5.

3. Pintér, K., Domán, M., Wehmann, E., Makrai, L., Magyar, T.: **Comparative analyses of phenotypic and genotypic antibiotic susceptibility of *Pasteurella multocida,*** The 7th Central European Forum for Microbiology, Siófok, Magyarország, 2025. október 6-8.

4. Pintér, K., Pollák, B. D., Makrai, L., Magyar, T.: **Különböző állatfajokból izolált *Pasteurella multocida* törzsek fenotípusos antibiotikum-érzékenységi vizsgálata**, Akadémiai Beszámolók, 2024. 01. 30.

5. Pintér, K., Pollák, B. D., Wehmann, E., Makrai, L., Solymosi, N., Magyar, T.: ***Pasteurella multocida* törzsek genotípusos jellemzése**, Akadémiai Beszámolók, 2024. 01. 30.

6. Pintér, K., Pollák, B. D., Domán, M., Wehmann, E., Makrai, L., Solymosi, N., Magyar, T.: **Virulenciagének előfordulási mintázata különböző gazdafajokból izolált *Pasteurella***

***multocida* törzsekben**, Akadémiai Beszámolók, Budapest, 2025. 01. 21.

7. Pintér, K., Domán, M., Wehmann, E., Makrai, L., Magyar T.: **A *Pasteurella multocida* genotípusos jellemzése, illetve a *Pasteurellaceae* család néhány tagjának antibiotikum-érzékenysége,** EKÖP Konferencia, Budapest, 2025. május 13.

8. Pintér, K., Domán, M., Wehmann, E., Makrai, L., Magyar T.: ***Pasteurella multocida* törzsek fenotípusos és genotípusos antibiotikum-érzékenységi profiljának összehasonlítása,** XXVIII. Tavaszi Szél Konferencia, Budapest, 2025. június 20-22.

9. Pintér, K., Domán, M., Wehmann, E., Magyar T.: ***Pasteurella multocida* törzsek fenotípusos és genotípusos jellemzői közötti összefüggések vizsgálata statisztikai elemzések segítségével,** Akadémiai Beszámolók, Budapest, 2026. 01. 27.